Adherence of *Borrelia burgdorferi* to the Proteoglycan Decorin

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Lyme disease is a tick-borne infection that can develop into a chronic, multisystemic disorder. The causative agent, *Borrelia burgdorferi*, is initially deposited by the tick into the host dermis, where it associates with collagen fibers, replicates, and eventually disseminates to other tissues. We have examined the adherence of the spirochete to different components of the collagen fiber and demonstrated that decorin, a proteoglycan which decorates collagen fibers, can support the attachment of *B. burgdorferi*. No significant direct attachment to isolated type I or III collagens could be detected. Attachment of the spirochetes to decorin was highly specific, and the process could be inhibited by soluble decorin but not by various unlabeled, unrelated components. *B. burgdorferi* also bound soluble ^{125}I-labeled decorin in a time- and concentration-dependent manner. Spirochete binding of soluble ^{125}I-labeled decorin required intact proteoglycan and could not be inhibited by either isolated core protein or glycosaminoglycan chain. *B. burgdorferi* expresses two decorin-binding proteins with apparent molecular masses of 19 and 20 kDa as revealed in a Western blot (immunoblot)-type assay. Our results indicate that decorin may mediate the adherence of *B. burgdorferi* to collagen fibers in skin and other tissues.

Lyme disease is a tick-borne infection caused by the spirochete *Borrelia burgdorferi* and other closely related borrelias. The disease is transmitted through the bite of a tick which attaches itself to the host and, upon feeding, deposits the spirochetes into the dermis of the host skin. There, *B. burgdorferi* replicates before endovascular dissemination to other organs. Typically, an annular, spreading skin lesion, erythema migrans, forms from the site of the tick bite. Early symptoms of Lyme disease are flu-like and may include fatigue and lethargy (25). Left untreated, Lyme disease can develop into a chronic, multisystemic disorder involving the skin, joints, heart, and central nervous system (3, 25, 26).

Once deposited in the dermis, the spirochetes become associated with and appear to colonize the collagen fibers (12, 27). The association of *B. burgdorferi* with collagen fibers in the dermis may be an important early event in the pathogenesis of Lyme disease. In mice, intradermal inoculation requires the fewest number of spirochetes to induce disease as compared with the intraperitoneal and gavage (force-feeding) routes (4), suggesting that the route of dermal entry is of importance for the disease process. Skin is also the most consistent tissue from which spirochete-positive cultures can be obtained (6). In persistent infection, the skin may provide a protective niche for replication, thereby acting as a reservoir of spirochetes for subsequent distribution to other tissues.

As *B. burgdorferi* disseminates to other organs, the organisms appear to localize to the extracellular spaces of these tissues as well. In several organs, including tendon (6, 7), ligament (15), heart (30), and muscle (5, 12), *B. burgdorferi* spirochetes are found primarily in close association with collagen fibers, suggesting that this association is an important mecha-

nism of tissue adherence in different stages of infection. Although the association of *B. burgdorferi* with collagen fibers has been reported previously by several investigators, the molecular mechanism responsible is not known.

The collagen network in skin is composed predominantly of collagens type I and type III. Decorin is a small dermatan sulfate proteoglycan which decorates collagen fibers in the extracellular matrix (13, 22, 24). In vitro, decorin has been shown to bind different collagen types and is believed to act as a regulator of collagen fiber formation. The proteoglycan can be isolated from many different tissues, such as skin, cartilage, and tendon. Decorin consists of a 36-kDa core protein, a single, serine-linked glycosaminoglycan (GAG) chain of the chondroitin sulfate or dermatan sulfate type, and up to three N-linked oligosaccharides (9, 23). GAGs are unbranched polysaccharides consisting of repeating disaccharide units. They are highly sulfated and therefore highly negatively charged. Decorin can inhibit transforming growth factor beta activity (29) and inactivate the complement component C1q (18) and has been proposed to act as an anti-inflammatory agent through these interactions.

In this article, we report that *B. burgdorferi* adheres to decorin-containing substrata, suggesting a possible mechanism of spirochete association with collagen fibers. *B. burgdorferi* N40 adheres to the collagen-associated proteoglycan decorin but does not adhere directly to type I or type III collagens. *B. burgdorferi*-decorin binding is specific and requires intact proteoglycan rather than isolated core protein or GAG chain. *B. burgdorferi* N40 expresses two decorin-binding proteins with apparent molecular masses of 19 and 20 kDa as revealed by Western blot (immunoblot)-type analysis.

MATERIALS AND METHODS

Bacterial strains and culture. Low-passage (fewer than 10 in vitro passages) *B. burgdorferi* N40 was used for all experiments unless specified otherwise. High-passage *B. burgdorferi* B31 (ATCC 35210) has undergone numerous in vitro passages. *B. burgdorferi* was cultured in BSK II medium at 34°C (2). Cultures

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were incubated in a GasPak chamber (BBL, Baltimore, Md.) with 3 to 6% O_2 until the cells reached the mid- to late-log phase. Cells were harvested by centrifugation at $14,500 \times g$ for 30 min and gently washed in sterile, filtered phosphate-buffered saline (PBS; pH 7.4; 0.137 M NaCl, 3 mM KCl, 4 mM Na_2HPO_4, 1 mM KH_2PO_4) three times. The spirochetes were resuspended in PBS, and the cell density was adjusted to 10^9 organisms per ml by use of a reference standard curve relating the A_{600} to the organism number as determined by dark-field microscopy. The spirochetes were stored at 4°C and retained decorin-binding activity for up to 1 month.

Staphylococcus aureus Phillips (clinical osteomyelitis isolate) and PH100 (collagen adhesin-negative isogenic mutant of strain Phillips) were grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) overnight at 37°C without antibiotics (21). The cells were washed and resuspended in PBS.

Labeling of decorin. Bovine decorin from fetal skin was purified as described previously (9). Decorin was labeled with NHS-LC-Biotin (Pierce, Rockford, Ill.) as described in the manufacturer's directions and stored at −20°C.

Decorin was iodinated by the chloramine T method as described by Hunter (16). Five microliters (0.5 mCi) of $Na^{125}I$ [Amersham Life Science, Arlington Heights, Ill.] was used to label 100 μg of decorin in 1 ml of PBS. The specific activity of the radiolabeled proteoglycan was estimated to be approximately 2×10^6 cpm/μg.

Attachment assay. Immulon-1 microtiter plate wells (Dynatech Labs, Chantilly, Va.) were coated with decorin, collagen type I from rat tail (Collaborative Biomedical Products, Bedford, Mass.), or collagen type III from calf skin (Sigma Chemical Co., St. Louis, Mo.). Decorin was dissolved in PBS, and collagens type I and III were dissolved in 20 mM acetic acid and adjusted to a concentration of 1 mg/ml. Two micrograms of each protein in a total volume of 50 μl was incubated in the microtiter plate wells at 4°C overnight. The wells were decanted and washed with 200 μl of PBS containing 0.1% bovine serum albumin (BSA) three times for 5 min each. Additional protein-binding sites were blocked by incubating the microtiter wells with 100 μl of a 1-mg/ml concentration of BSA in PBS for 2 h. The wells were washed and incubated for 1 h with 25 μl of a suspension containing 10^9 organisms per ml of PBS–0.1% BSA. After washing the wells to remove unattached bacteria, the wells were incubated for 1 h with 100 μl of a 1:1,000 dilution of anti-*B. burgdorferi* rabbit serum (rabbits were inoculated with 10^8 organisms of washed *B. burgdorferi* B31 per ml, and serum was collected 3 weeks postinoculation) in PBS–0.1% BSA. This step was omitted when assaying *S. aureus* attachment because protein A on the surface of *S. aureus* binds the secondary antibody directly. The wells were washed and subsequently incubated with 100 μl of a 1:1,000 dilution of goat anti-rabbit alkaline phosphatase conjugate (Bio-Rad, Hercules, Calif.) in PBS–0.1% BSA for 1 h and then washed and incubated with 100 μl of a 1-mg/ml concentration of Sigma 104 phosphatase substrate dissolved in 1 M diethanolamine–0.5 mM $MgCl_2$ (pH 9.8) at 37°C for 30 to 45 min. The A_{405} was determined in a microplate reader (Molecular Devices, Menlo Park, Calif.).

To assay inhibition of attachment, 100-μl suspensions containing 10^9 organisms of *B. burgdorferi* N40 per ml were preincubated with 2 μg of the potential competitor (or as otherwise stated) for 1 h at room temperature. The potential competitors included decorin, BSA (The Binding Site, San Diego, Calif., or ICN, Costa Mesa, Calif.), fetuin type IV (Sigma), thyroglobulin type II (Sigma), fibrinogen (KabiVitrium, Stockholm, Sweden), aggrecan (isolated from bovine cartilage), heparin (Sigma), and chondroitin sulfate type A (from whale and shark cartilage; Sigma). One microliter of 10% BSA was added to obtain a final concentration of 1% BSA. The suspensions were added to protein-coated microtiter wells, and the assay was continued as described above.

Binding assay. *B. burgdorferi* N40 cells (1.5×10^8) were incubated with approximately 50,000 cpm of ^{125}I-labeled decorin in a final volume of 0.5 ml of PBS containing 1% BSA for 1 h at room temperature. The reaction was stopped by the addition of 3 ml of PBS containing 1% BSA; centrifugation at $6,000 \times g$ for 30 min followed. Radiolabeled decorin associated with the bacterial pellet was quantitated in a Cobra II Auto-Gamma Counter (Packard Instruments, Meriden, Conn.). Radioactivity recovered in tubes incubated as described above, but without bacteria, was regarded as background and subtracted from the values obtained with bacteria. Time dependence of binding was assayed by incubating *B. burgdorferi* with ^{125}I-labeled decorin as described above and stopping the reaction at the specified times.

Inhibition of binding was assayed by preincubating washed *B. burgdorferi* N40 (10^8 organisms per ml) with 5 μg of unlabeled competitor for 30 min. Radiolabeled decorin (50,000 cpm) was added, and the incubation was continued for another 30 min. The reaction was stopped by the addition of 3 ml of PBS containing 1% BSA, and the assay was continued as described above.

SDS-PAGE and Western blot-type assay. Proteins from *B. burgdorferi* whole-cell lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (19) and probed with a Western blot-type assay. For SDS-PAGE, 2×10^7 *B. burgdorferi* cells were lysed by boiling in SDS under reducing conditions and subjected to electrophoresis through a 5 to 15% gradient acrylamide slab gel at 175 V for 30 min. For Western blot-type assays, the proteins were transferred from the polyacrylamide gel to a nitrocellulose membrane (Schleicher & Schuell, Inc., Keene, N.H.) by electroblot for 1.5 h at 4°C. Additional protein-binding sites on the membrane were blocked by incubating in 3% nonfat dry milk in TBST (0.15 M NaCl, 20 mM Tris-HCl, 0.05% Tween 20 [pH 7.4]) for 2 h at room temperature or overnight at 4°C. The membrane was
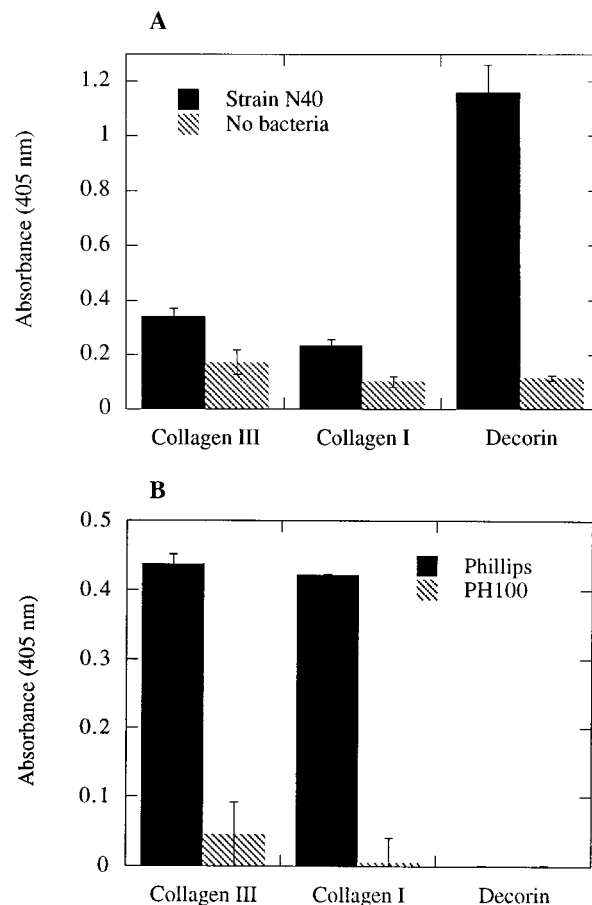

FIG. 1. Attachment of bacterial strains to microtiter wells coated with type III collagen, type I collagen, or decorin. Protein-coated microtiter wells were incubated with *B. burgdorferi* N40 or no bacteria (A) or *S. aureus* Phillips (collagen adhesin positive) or *S. aureus* PH100 (collagen adhesin negative) (B). Attachment to the substrate was quantitated by an ELISA. Error bars represent the standard deviations of three separate determinations.

incubated at room temperature with 0.1 μg of biotin-labeled decorin per ml of TBST for 1 h, washed, and incubated with a 1:3,000 dilution of avidin D horseradish peroxidase conjugate (Vector Laboratories, Burlingame, Calif.) in TBST for 1 h. The membrane was washed and incubated in 1 ml of Enhanced Chemi-Luminescence detection reagents 1 and 2 (Amersham Life Science) for 1 min and exposed to x-ray film for 1 to 5 s.

RESULTS

B. burgdorferi **adheres to decorin.** To determine whether any of the major macromolecular components of the dermal collagen fibers (i.e., collagens type I and III and decorin) can support the adherence of *B. burgdorferi*, we used an in vitro attachment assay. Microtiter wells were coated with decorin or collagens, and a suspension of spirochetes was incubated for 1 h in the protein-coated wells. Adherent spirochetes were detected by an immunological method after nonadherent organisms were removed by washing. *B. burgdorferi* N40 adhered to wells coated with decorin (Fig. 1A), whereas spirochete adherence to collagen-coated wells was only marginally greater than adherence to BSA-coated control wells (data not shown). Furthermore, in this enzyme-linked immunosorbent assay (ELISA)-type test, the signals from the collagen-coated wells incubated with *B. burgdorferi* were comparable to those from protein-coated wells incubated in the absence of bacteria (Fig. 1A). As a control, we demon-
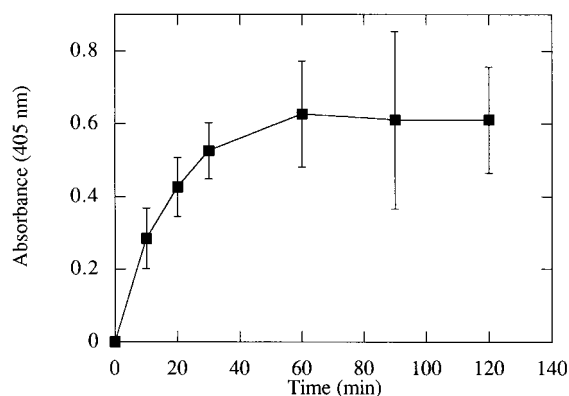
FIG. 2. Time dependence of the attachment of *B. burgdorferi* N40 to decorin substrata. *B. burgdorferi* was incubated in decorin-coated microtiter wells for various time intervals. Attachment to the substrate was quantitated by an ELISA. Error bars represent the standard deviations of three separate determinations.

FIG. 4. Specificity of the attachment of *B. burgdorferi* N40 to decorin substrata. *B. burgdorferi* was incubated with various potential inhibitors before being transferred to decorin-coated microtiter wells. Potential inhibitors were BSA, decorin (DCN), aggrecan (AGN), fetuin (FTN), thyroglobulin (TGN), chondroitin sulfate type A (CSA), and fibrinogen (FG). Attachment to the substrate was quantitated by an ELISA. Error bars represent the standard deviations of three separate determinations.

strated (Fig. 1B) that the collagen-coated wells could support adherence of *S. aureus* Phillips, which expresses a collagen adhesin, but could not support adherence of strain PH100, a collagen adhesin-negative mutant. These data demonstrate that *B. burgdorferi* N40 adhered to decorin but not to the other main components of dermal collagen fibers.

When the adherence of *B. burgdorferi* to decorin-coated microtiter wells was assayed as a function of time, a time-dependent process in which maximal adherence was reached at ~1 h was observed (Fig. 2). Continuing the incubation for another hour did not result in an increased number of adhering bacteria.

B. burgdorferi also appears to recognize soluble decorin. Preincubation of the spirochetes with increasing concentrations of soluble decorin resulted in a progressively reduced adherence to the decorin substrate. A 50% reduction in attachment was observed when 2.5×10^7 spirochetes were preincubated with 0.1 μg of decorin (Fig. 3).

To determine the specificity of *B. burgdorferi* attachment to decorin-coated wells, we attempted to inhibit such attachment by preincubation of the spirochetes with various soluble extracellular components, such as fetuin, thyroglobulin, fibrinogen, aggrecan, and chondroitin sulfate chains. *B. burgdorferi* was
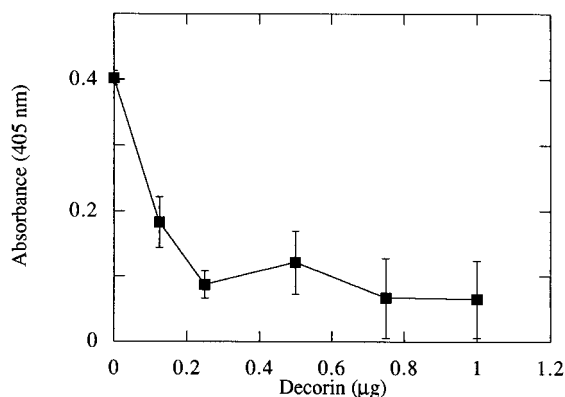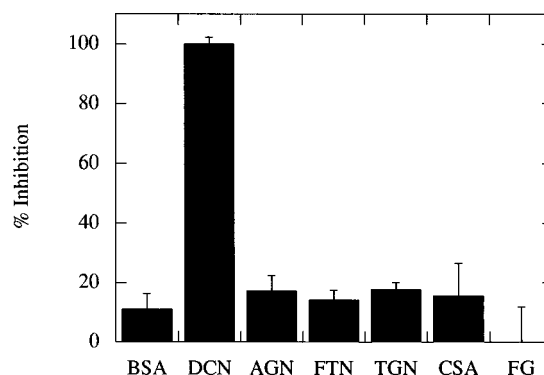

FIG. 3. Inhibition of the attachment of *B. burgdorferi* N40 to decorin substrata. *B. burgdorferi* was incubated with increasing concentrations of decorin before being transferred to decorin-coated microtiter wells. Attachment to the substrate was quantitated by an ELISA. Error bars represent the standard deviations of three separate determinations.

preincubated with each component individually for 1 h, and the suspensions were transferred to microtiter wells where the spirochetes were allowed to attach to decorin-coated wells. Decorin inhibited attachment by 100%, whereas the other potential inhibitors only marginally affected attachment of the spirochetes, resulting in less than 20% inhibition (Fig. 4). *B. burgdorferi* attachment to decorin thus appears to be highly specific.

B. burgdorferi binds soluble ^{125}I-labeled decorin. We used a modified in vitro binding assay to determine whether *B. burgdorferi* binds to soluble ^{125}I-labeled decorin. Spirochetes were incubated in a suspension containing PBS, 1% BSA, and ^{125}I-labeled decorin. At the end of the incubation, the bacteria were collected by centrifugation and the amount of decorin bound was assayed by measuring radioactivity in the pellet.

When binding was assayed as a function of time from 0 to 120 min, maximum binding was achieved in 15 min and remained constant for up to 2 h (Fig. 5). Prolonged incubation for 3 to 4 h often resulted in a decrease of binding (data not shown). A high-passage isolate of *B. burgdorferi* B31 showed no binding at any time points (Fig. 5).

To correlate these results with the results of the attachment assay, we attempted to inhibit *B. burgdorferi* binding of ^{125}I-labeled decorin with different unlabeled components in suspension. The spirochetes were preincubated with the unlabeled potential competitor for 30 min in the absence of radiolabeled decorin. After the addition of ^{125}I-labeled decorin, the incubation was continued for another 30 min. Decorin inhibited the binding of the radiolabeled ligand by 63%, whereas the other potential inhibitors tested (aggrecan, thyroglobulin, BSA, fetuin, chondroitin sulfate, fibrinogen, and heparin) were essentially without effect, all reducing binding by less than 10% (Fig. 6). Thus, the effect of the unlabeled inhibitors on bacterial binding of soluble ^{125}I-labeled decorin is similar to that on the attachment of *B. burgdorferi* to decorin substrates, indicating that the same bacterial molecule(s) is involved.

In attempts to determine which domain of decorin is involved in binding to the spirochete, we tried to inhibit *B. burgdorferi* binding to intact ^{125}I-labeled decorin by use of isolated core protein, isolated GAG chain, or an equimolar mixture of both (8). Binding was inhibited by intact proteoglycan (containing the GAG chain covalently attached to the core
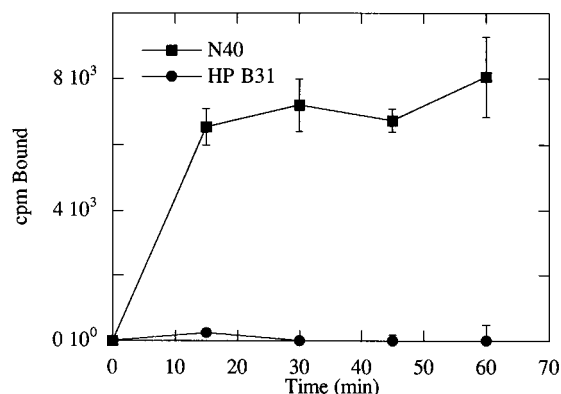
FIG. 5. Time dependence of the binding of *B. burgdorferi* to soluble decorin. *B. burgdorferi* N40 and HP B31 (high passage) were incubated with decorin for various time intervals. Binding to decorin was determined by measuring binding of ^{125}I-labeled decorin. Error bars represent the standard deviations of three separate determinations.

protein) but was not inhibited by isolated core protein, isolated GAG chain, or a mixture of both (Fig. 7).

When *B. burgdorferi* was incubated with increasing amounts of ^{125}I-labeled decorin, we observed a concentration-dependent binding of the ligand (Fig. 8). Radioactivity recovered in tubes incubated in the absence of bacteria (nonspecific binding) also increased as the amount of labeled decorin increased. Specific bacterial binding (total binding minus nonspecific binding) of ^{125}I-labeled decorin appeared to approach saturation. From these data, we estimated an approximate K_d value for the interaction as well as the number (n) of decorin-binding sites per bacteria. To this end, $[S]_{bound}/[S]_{free}$ was plotted versus $[S]_{bound}$, where the substrate, S, is decorin. From this plot, the K_d was calculated to be approximately 3×10^{-7} M^{-1}, indicating a moderate affinity, and n was calculated to be approximately 5×10^4 decorin-binding sites per organism, indicating a small copy number (data not shown). Although the standard deviation for decorin bound (Fig. 8) is large, we present these values as rough estimates of K_d and n.

Identification of decorin-binding proteins from *B. burgdorferi*. In an attempt to identify distinct decorin-binding proteins

FIG. 7. Involvement of core protein and GAG chain in decorin binding. *B. burgdorferi* N40 was incubated with unlabeled intact decorin, isolated core protein, isolated GAG chain, and a mixture of isolated core and GAG before being incubated with radiolabeled intact decorin. Binding of bacteria to decorin in the presence of potential inhibitors was quantitated by measuring ^{125}I-decorin bound. All datum points were measured in triplicate.

expressed by *B. burgdorferi*, we used a Western blot-type assay with biotin-labeled decorin as the probe. Proteins from whole-cell *B. burgdorferi* lysates were separated by SDS-PAGE under reducing conditions and transferred to a nitrocellulose membrane. After blocking additional protein-binding sites with a solution containing 3% nonfat dry milk, biotin-labeled decorin was allowed to bind to proteins on the membrane, followed by binding of horseradish peroxidase-conjugated avidin to the biotin-labeled decorin. Decorin-binding proteins were visualized by chemiluminescence. This assay revealed the presence of two decorin-binding proteins with apparent molecular masses of 19 and 20 kDa in the mixture of proteins from *B. burgdorferi* N40 (Fig. 9). SDS-PAGE followed by staining with Coomassie brilliant blue indicated that these proteins constitute a small portion of the total proteins of *B. burgdorferi*. The two decorin-binding proteins run directly beneath OspC (the prominent band at ~21 kDa) but are hardly visible, if at all, by
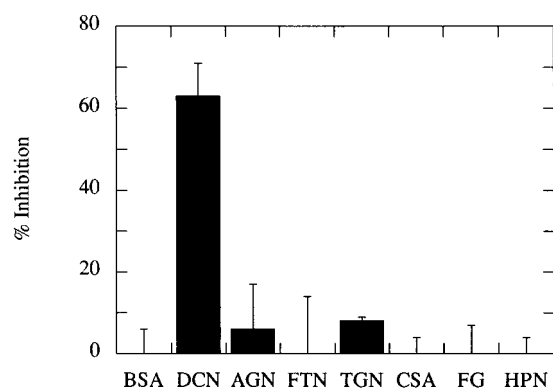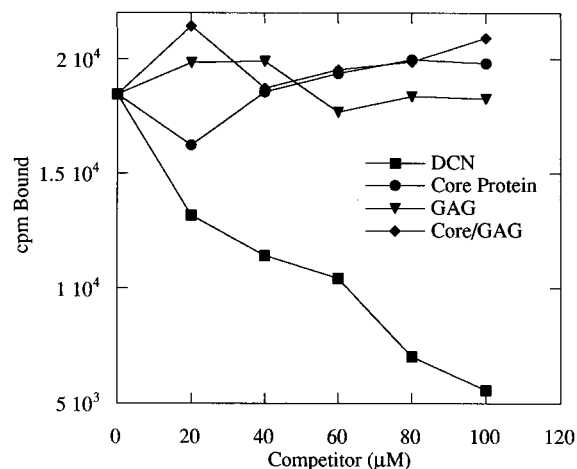

FIG. 6. Specificity of the binding of *B. burgdorferi* N40 to soluble decorin. *B. burgdorferi* was incubated with various potential inhibitors before being allowed to bind to decorin. Potential inhibitors were BSA, decorin (DCN), aggrecan (AGN), fetuin (FTN), thyroglobulin (TGN), chondroitin sulfate type A (CSA), fibrinogen (FG), and heparin (HPN). Binding to decorin was determined by measuring binding of ^{125}I-labeled decorin. Error bars represent the standard deviations of three separate determinations.
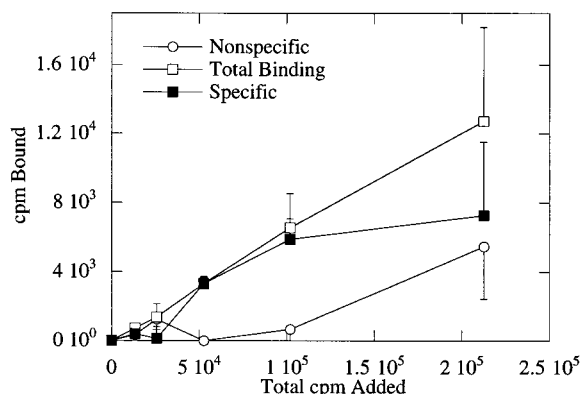

FIG. 8. Saturation of decorin binding to *B. burgdorferi* N40. *B. burgdorferi* was incubated with increasing concentrations of radiolabeled decorin in the presence and absence of excess unlabeled decorin. The binding of *B. burgdorferi* to ^{125}I-labeled decorin in the presence (nonspecific binding) and absence (total binding) of excess unlabeled decorin was measured. Specific binding was calculated by subtracting nonspecific binding from total binding. Error bars represent the standard deviations of three separate determinations.
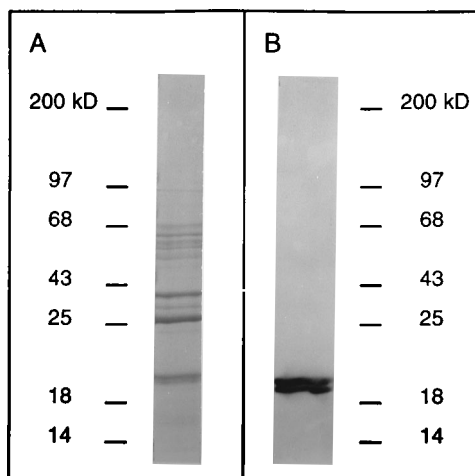
FIG. 9. Identification of decorin-binding proteins of *B. burgdorferi* N40. *B. burgdorferi* whole-cell lysate was subjected to SDS-PAGE (5 to 15%) under reducing conditions and stained with Coomassie brilliant blue (A) or transferred to a nitrocellulose membrane (B). After blocking additional protein-binding sites, proteins on the membrane were probed with biotin-labeled decorin and visualized by chemiluminescence. The migration of standard proteins with known molecular masses (in kilodaltons) are shown on the left and right.

Coomassie blue stain. When proteins from the high-passage, non-decorin-binding B31 strain were analyzed in the same manner, no decorin-binding protein(s) could be detected.

We also attempted to inhibit binding of biotin-labeled decorin to *B. burgdorferi* proteins in the Western blot-type assay. We preincubated the membrane with the same unlabeled proteins as those used in attempts to block bacterial attachment to decorin substrate or to inhibit the binding of ^{125}I-labeled decorin to intact spirochetes (data not shown). The same type of specificity was observed in all three assays. Furthermore, the presence of isolated GAG chain or core protein did not interfere with binding of biotin-labeled decorin to *B. burgdorferi* proteins. Taken together, these data suggest that the 19- and 20-kDa proteins identified as decorin-binding proteins are responsible for binding of ^{125}I-labeled decorin to intact spirochetes and mediate adherence of bacteria to decorin substrates.

DISCUSSION

Previous studies have revealed that *B. burgdorferi* is predominantly an extracellular pathogen and that the spirochetes are often found in intimate association with collagen fibers (5–7, 12). On the basis of this association, we hypothesized that *B. burgdorferi* may express adhesins that specifically recognize a component of collagen fibers. In the skin, a tissue where *B. burgdorferi* is consistently found, the collagen network is composed mainly of collagen types I and III and the proteoglycan decorin, which is associated with the collagen fibers.

B. burgdorferi adhered to substrata composed of decorin but did not adhere to collagens type I or III. These data suggest that decorin is a possible target for *B. burgdorferi* adherence in the skin. Previously, *B. burgdorferi* adherence to heparin (17) and $\alpha_{IIb}\beta_3$ integrin (10, 11) has been reported. Furthermore, heparin inhibits the adherence of *B. burgdorferi* to cultured HeLa cells (17) but, as shown in this study, does not affect the binding of decorin to the spirochete. *B. burgdorferi* most likely possesses several mechanisms of host tissue adherence; however, it is unlikely that any of the previously described adherence mechanisms are responsible for the observed colonization of collagen fibers in the dermis.

B. burgdorferi also binds to soluble decorin in a process that exhibits saturation kinetics and occurs in a time- and concentration-dependent manner. Maximal binding was achieved more quickly when decorin was in solution than when it was immobilized onto microtiter wells. The reason for this discrepancy is unknown. Both the binding of soluble ^{125}I-labeled decorin and the attachment of spirochetes to a decorin substrate were effectively inhibited by decorin. Other extracellular matrix proteins had only marginal effects, suggesting a high degree of specificity. Furthermore, neither isolated core protein nor GAG chain alone or in combination could inhibit binding.

The *Borrelia* binding site on the decorin molecule has not been identified. Presumably, decorin binds both collagen and borrelias at once, with the two interactions involving different sites on the proteoglycan. The requirement of intact decorin for binding to *B. burgdorferi* suggests that the decorin adhesin on the spirochete may recognize a conformational motif that is destroyed upon separation of the core protein and the GAG chain.

We have estimated the K_d for the binding of *B. burgdorferi* to decorin to be approximately 3×10^{-7} M^{-1}, indicating moderate affinity. The number of binding sites, n, was calculated to be approximately 5×10^4 copies per organism, which is a low copy number. SDS-PAGE analysis also seems to indicate that the decorin-binding proteins are not abundant *B. burgdorferi* proteins.

By Western blot-type assay, we have identified two putative decorin adhesins with apparent molecular masses of 19 and 20 kDa. At this point, it is not clear whether the 19-kDa protein is a truncated product derived from the same gene as the 20-kDa protein or whether the two proteins are genetically distinct.

The data presented here demonstrate that two proteins expressed by *B. burgdorferi* N40 may act as adhesins mediating attachment of spirochetes to dermal collagen via decorin. Several other *B. burgdorferi* strains, including low-passage Sh-2-82 (*Ixodes dammini* tick isolate from Shelter Island, N.Y.), B31 (2), and 297 (17), also bind ^{125}I-labeled decorin and express a decorin-binding protein(s) in the 20-kDa molecular mass range (data not shown). Studies in our laboratory are under way to compare the structures of decorin-binding proteins from different strains and to assess their roles as virulence factors.

The adherence of microbes to various cell-surface and extracellular matrix components has been widely reported (1, 11, 14, 17, 20, 28); however, this is the first report of microbial attachment to the proteoglycan decorin. Binding to decorin may serve as an adhesion mechanism which allows the spirochetes to gain a foothold in the host tissue upon entering the skin. The *B. burgdorferi*-decorin interaction may also play a role in the inflammatory process which is characteristic of Lyme disease. Decorin is thought to act as a regulator of inflammation through its interaction with C1q (18) and transforming growth factor beta (29). *B. burgdorferi* adherence to decorin may affect the inflammatory process through these interactions.

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